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## **Evaluating Popcorn as a Potential Refuge of *Ostrinia nubilalis* (Lepidoptera: Crambidae)**

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# Evaluating Popcorn as a Potential Refuge of *Ostrinia nubilalis* (Lepidoptera: Crambidae)

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**ABSTRACT** Popcorn was evaluated in a series of experiments conducted over four growing seasons for its potential as a refuge for European corn borer, *Ostrinia nubilalis* (Hübner). Objectives of these studies were to determine whether more larvae were produced in popcorn than in field corn and to determine how popcorn influenced female oviposition and larval distribution in neighboring field corn. Two varieties of popcorn (M140, 105d and M3374Y, 118d), one mixture of popcorn (50% 105d and 50% 118d), and field corn (DK580, 108d) were evaluated. Number of egg masses, eggs per egg mass, and larvae were significantly higher in popcorn compared with field corn. Moth oviposition and larval distribution were evaluated using 105d popcorn embedded in several cornfields across Iowa. The row of field corn adjacent to popcorn had significantly more larvae compared with background field corn. In larger field experiments, *O. nubilalis* larval survival after overwintering was significantly different, with 2.2–18.7 times more *O. nubilalis* larvae surviving in popcorn than field corn. The potential use of popcorn as an *O. nubilalis* refuge for genetically engineered corn is considered.

**KEY WORDS** insect resistance management, genetically engineered corn, maize, European corn borer

Many growers are using genetically engineered (GE) *Bacillus thuringiensis* (*Bt*) hybrid corn, *Zea mays* L., to control European corn borer, *Ostrinia nubilalis* (Hübner). Commercially available *Bt* corn hybrids in the United States express either Cry1Ab or Cry1F proteins, which are sold as YieldGard and Herculex, respectively. The most serious threat to the long-term use of *Bt* corn is the evolution of resistance to *Bt* toxins by insect pests (Gould and Tabashnik 1998). The U.S. Environmental Protection Agency (EPA) has adopted an insect resistance management (IRM) strategy requiring high-dose plants and refuge (Tabashnik and Croft 1982, Roush 1989, 1994). Current *Bt* hybrids seem to satisfy high-dose requirements for *O. nubilalis* (Walker et al. 2000, U.S. EPA 2005), and the EPA requires planting 20% non-*Bt* corn refuge within 0.5 mi of *Bt* corn with activity against lepidoteran insects (U.S. EPA 2001).

Non-*Bt* corn refuges are a source of *O. nubilalis* susceptible to *Bt* corn. In theory, moths from refuge will outnumber the few resistant moths that may survive in *Bt* corn fields, decreasing the chance resistant moths will mate with each other. Matings between

resistant and susceptible moths dilute resistance genes and produce heterozygous offspring, which theoretically should not be able to survive the high dose of *Bt* toxin expressed in *Bt* corn (Tabashnik and Croft 1982, Zhao et al. 2005). Host plants, besides corn, commonly found in or near cornfields, do not produce adequate numbers of *O. nubilalis* to be considered a viable refuge option (Losey et al. 2001), so growers rely on refuge field corn. There are many types of corn, such as popcorn or varieties with tropical germplasm, which could be considered as refuge options if they produce more *O. nubilalis* than traditional field corn. Refuge for *Bt* corn should produce sufficient *O. nubilalis* moths whose flights coincide with each generation of the natural population (Ostlie et al. 1997), which is two generations per growing season for much of the Corn Belt. Most popcorn varieties are susceptible to *O. nubilalis* and potentially could produce many moths (Andrew and Carlson 1976, Wilson et al. 1993). High production of *O. nubilalis* could increase the IRM value of popcorn, and such a “super” refuge could open the door for reducing refuge percentages.

Producing large numbers of *O. nubilalis* in a small area under certain conditions could result in increased selection for resistance. There could be a high number of *O. nubilalis* larvae moving at the interface of popcorn and *Bt* corn (Davis and Onstad 2000), especially if larval movement is density dependent. Late instars of *O. nubilalis* moving from popcorn to *Bt* corn could violate the high-dose strategy if heterozygous resistant larvae move from popcorn and survive on *Bt* corn (Dulmage et al. 1978, Eborá et al. 1984, Mallet and

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Table 1. Maturities and varieties of popcorn and field corn treatments used in the experiments

| Treatment | Maturities  | Varieties                               |
|-----------|-------------|---|
| 1         | 108d        | Dekalb 580/5 (Field corn)               |
| 2         | 90d         | McHone 8368                             |
| 3         | 105d        | McHone 140                              |
| 4         | 118d        | McHone 3374Y                            |
| 5         | 90/105d     | McHone 8368/McHone 140                  |
| 6         | 90/118d     | McHone 8368/McHone 3374Y                |
| 7         | 105/118d    | McHone 140/McHone 3374Y                 |
| 8         | 90/105/118d | McHone 8368/McHone 140/<br>McHone 3374Y |

All treatments were evaluated in 2000. Treatments in bold were used from 2000 to 2002.

Porter 1992, Davis and Coleman 1997). Also, there are questions concerning female oviposition rates in popcorn versus *Bt* corn, which potentially could influence resistance development if sufficient numbers of moths are not produced in the refuge (Caprio 2001, Caprio et al. 2004).

Potential value of popcorn as an *O. nubilalis* refuge for *Bt* corn is considered in this paper with a series of experiments conducted over four growing seasons. Objectives were to compare *O. nubilalis* production in various types and mixtures of popcorn and nontransformed field corn; determine how popcorn might influence oviposition and larval movement; and determine whether *O. nubilalis* production in popcorn is affected by tillage practices, the endophytic fungus, *Beauveria bassiana* (Balsamo) Vuillemin, or the parasitoid *Macrocentrus cingulum* Reinhard (Hymenoptera: Braconidae).

Table 2. Farms, corn varieties, planting dates, and beginning pollination dates for popcorn evaluation and proximity experiments during each year (2000–2002)

| Farm                           | Variety  | Planting date | Pollination  |
|--------------------------------|--|---------------|--------------|
| Popcorn evaluation experiments |  |               |              |
| AG450                          | Pioneer hybrid 33A14 (Pioneer Hi-bred, Johnston, IA) | 29 April 2000 | 30 July 2000 |
| AG450                          | Field corn and popcorn treatments                    | 16 May 2000   | 9 Aug. 2000  |
| AG450                          | Wilson 615 (Wilson Genetics, Harlan, IA)             | 9 May 2001    | 7 Aug. 2001  |
| AG450                          | Field corn and popcorn treatments                    | 10 May 2001   | 7 Aug. 2001  |
| Ankeny                         | Novartis 59-Q9 (Novartis Seed, Golden Valley, Mn)    | 28 April 2002 | 30 July 2002 |
| Ankeny                         | Field corn and popcorn treatments                    | 9 May 2002    | 30 July 2002 |
| Proximity experiments          |  |               |              |
| AG450                          | Pioneer hybrid 33A14 (Pioneer Hi-bred, Johnston, IA) | 29 April 2000 | 30 July 2000 |
| AG450                          | McHone 140 Popcorn (McHone Seed, Ames, IA)           | 16 May 2000   | 9 Aug. 2000  |
| Ankeny                         | Pioneer hybrid 33A14 (Pioneer Hi-bred, Johnston, IA) | 29 April 2000 | 30 July 2000 |
| Ankeny                         | McHone 140 Popcorn (McHone Seed, Ames, IA)           | 24 May 2000   | 16 Aug. 2000 |
| Englebrecht                    | Garst 8464 (Garst Seed, Slater, IA)                  | 29 April 2000 | 30 July 2000 |
| Englebrecht                    | McHone 140 Popcorn (McHone Seed, Ames, IA)           | 3 June 2000   | 16 Aug. 2000 |
| AG450                          | Wilson 615 (Wilson Genetics, Harlan, IA)             | 9 May 2001    | 7 Aug. 2001  |
| AG450                          | McHone 140 Popcorn (McHone Seed, Ames, IA)           | 10 May 2001   | 7 Aug. 2001  |
| Ankeny                         | Garst 8342 (Garst Seed, Slater, IA)                  | 10 May 2001   | 7 Aug. 2001  |
| Ankeny                         | M140 Popcorn (McHone Seed, Ames, IA)                 | 17 May 2001   | 14 Aug. 2001 |
| Englebrecht                    | Garst 8464 (Garst Seed, Slater, IA)                  | 29 April 2001 | 30 July 2001 |
| Englebrecht                    | McHone 140 Popcorn (McHone Seed, Ames, IA)           | 6 May 2001    | 30 July 2001 |
| Atomic Bottom                  | Wilson 615 (Wilson Genetics, Harlan, IA)             | 3 May 2002    | 30 July 2002 |
| Atomic Bottom                  | McHone 140 Popcorn (McHone Seed, Ames, IA)           | 4 May 2002    | 30 July 2002 |
| Ankeny                         | Novartis 59-Q9 (Novartis Seed, Golden Valley, Mn)    | 28 April 2002 | 30 July 2002 |
| Ankeny                         | McHone 140 Popcorn (McHone Seed, Ames, IA)           | 9 May 2002    | 30 July 2002 |
| Englebrecht                    | Garst 8464 (Garst Seed, Slater, IA)                  | 4 May 2002    | 30 July 2002 |
| Englebrecht                    | McHone 140 Popcorn (McHone Seed, Ames, IA)           | 12 May 2002   | 30 July 2002 |

Materials and Methods

Numbers of *O. nubilalis* found in three varieties of popcorn, mixtures of these varieties, and field corn were compared in 2000. Two varieties of popcorn, a mixture of these varieties, and field corn were compared in 2001 and 2002. In other experiments, distributions of egg masses and larvae in and near popcorn were measured in several fields in Iowa in 2000, 2001, and 2002. The percentage of overwintering *O. nubilalis* larvae surviving in field corn and popcorn refuge was evaluated in 2001, 2002, and 2003.

**Popcorn Evaluations.** Field tests to assess *O. nubilalis* production in three varieties of popcorn, four mixtures of these varieties, and field corn were conducted on the Iowa State University (ISU) AG 450 Farm in 2000. Two varieties of popcorn and a popcorn mixture of these varieties were compared with field corn at the ISU AG450 Farm (2001) and ISU Ankeny Research Farm (2002) (Tables 1–2). Each year the treatments were put into five randomized complete blocks; plots were 9 by 9 m. Treatments were hand planted at a rate of  $\approx$ 110,000 seeds/ha.

Five random, square meter counts were taken in each plot to estimate plant population. During the first *O. nubilalis* generation (2001–2002) and second generation (2000–2002), 10 plants were dissected in each plot to determine larval densities. Plants dissected for *O. nubilalis* larvae were between V12 and VT stages of development during the first generation and R4 and R6 stages of development during the second generation (Ritchie et al. 1997).

**Popcorn and Field Corn Proximity Experiments.** Field corn was planted in fields measuring  $>20$  ha on ISU farms AG450, Ankeny, and Atomic Bottom, and

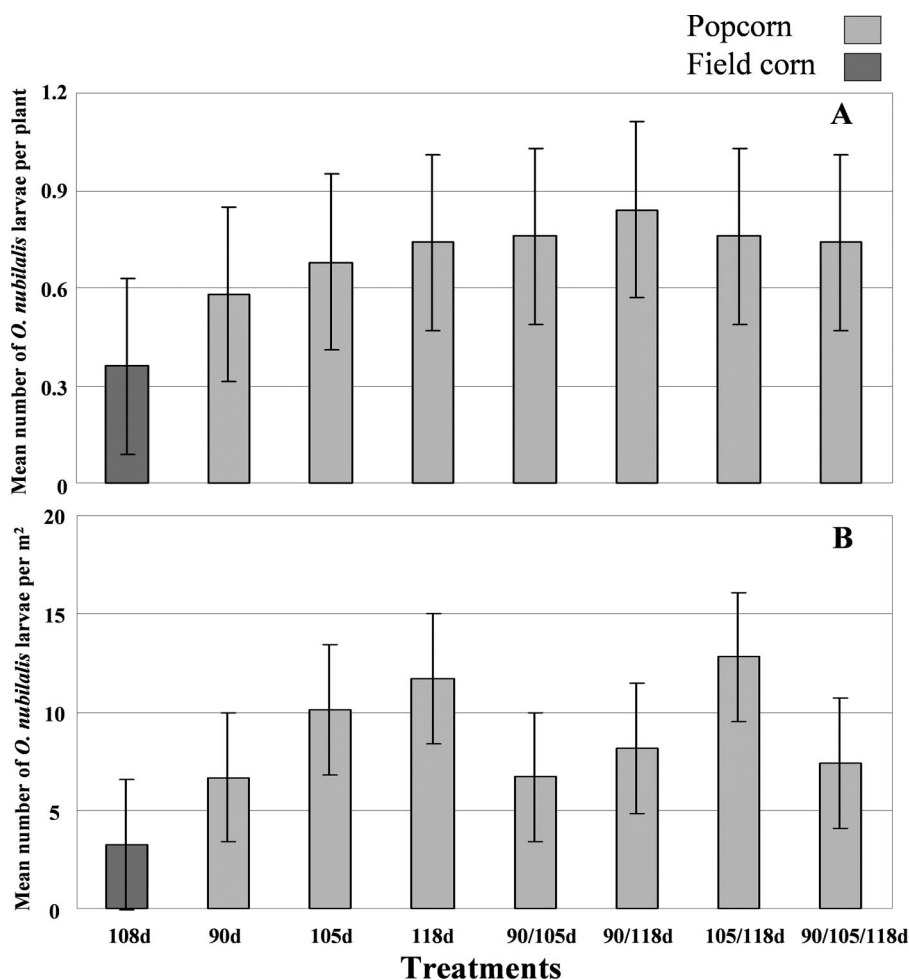


Fig. 1. (A) Mean number  $\pm$  SE of second-generation *O. nubilalis* larvae found per plant in popcorn and field corn in 2000 ( $F = 0.71$ ;  $df = 7,28$ ;  $P = 0.72$ ). (B) Mean number  $\pm$  SE of second-generation *O. nubilalis* larvae found per square meter in popcorn and field corn in 2000 ( $F = 1.13$ ;  $df = 7,28$ ;  $P = 0.38$ ). Day is represented by d.

one private farm, Englebrecht, at  $\approx 74,000$  seeds/ha (Table 2). Three 9 by 9-m popcorn (105d) plots, separated by 30 m, were hand planted within each field at a rate of 110,000 seeds/ha. Samples were taken in four cardinal directions from each popcorn plot at 0 (popcorn edge), 0.8, 1.5, 3, 6, and 12 m (1, 2, 4, 8, and 16 rows). During the second *O. nubilalis* generation (2001–2002), five plants were examined for egg masses at each sampling location, and number of eggs in each egg mass was recorded. Before harvest, five plants at each sampling location were dissected and inspected for larvae. These data were used to determine egg mass and larval distributions in popcorn and adjacent field corn and to estimate size of egg masses along transects.

**Small Field Experiments.** Field corn and popcorn were planted into three 18 by 36-m fields on the Ankeny farm. One half of each field was planted to field corn ( $\approx 74,000$  seeds/ha), and the remaining half was planted to popcorn ( $\approx 110,000$  seeds/ha). No-till or conventional tillage practices were used to manage

six (5.5 by 18 m) randomly selected plots within each field. Corn plants were allowed to reach maturity but were not harvested. The following spring, three random square meter samples of corn stalks were taken from each plot to evaluate survival of overwintering *O. nubilalis* larvae. Corn stalks were placed in black plastic bags and taken to the laboratory, where they were stored at 4°C until processed.

Numbers of live and dead larvae were recorded. Live larvae were placed in two dram shell vials (Bio-Quip Products, Gardenia, CA) and stored in Percival Scientific (model I-35VL; Boone, IA) environmental chambers at 26°C, 80% RH, and a photoperiod of 16:8 (L:D) h to complete development. Parasitoids from parasitized larvae were stored in vials at 0°C. Dead larvae also were placed in vials and stored at 0°C. Later, the thoraces of up to eight dead larvae were placed on a selective medium (Doberski and Tribe 1980) to determine whether death was caused by *B. bassiana* infection.

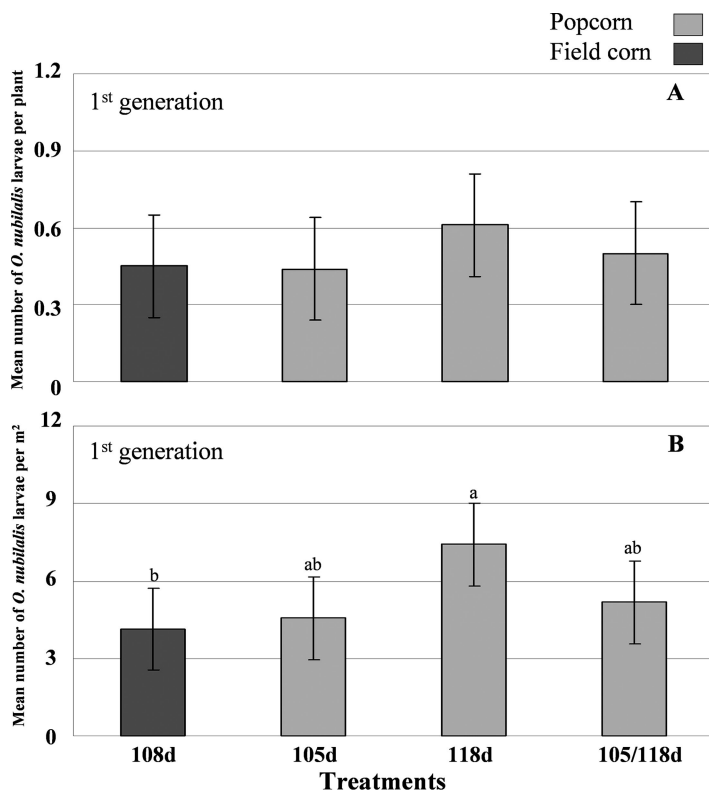


Fig. 2. (A) Mean number  $\pm$  SE of first-generation *O. nubilalis* larvae found per plant in popcorn and field corn 2000–2002. Treatment means were not significantly different ( $F = 1.58$ ;  $df = 3,27$ ;  $P = 0.22$ ). (B) Mean number  $\pm$  SE of first-generation *O. nubilalis* larvae found per plant in popcorn and field corn 2000–2002. Treatment means were significantly different ( $F = 7.19$ ;  $df = 3,6$ ;  $P = 0.02$ ). Bars marked with the same letter are not significantly different (ANOVA,  $P \leq 0.05$ ; Tukey-Kramer,  $\alpha = 0.05$ ). Day is represented by d.

**Analyses.** Second-generation *O. nubilalis* larval production (per plant and per square meter) from seven popcorn treatments and one field corn treatment in 2000 was analyzed with analysis of variance (ANOVA; Proc Mixed; SAS Institute 1990). Corn treatment was treated as a fixed effect, and block was treated as a random effect. In 2000–2002, larval production from four treatments (105d, 118d, mixture popcorn, and field corn) was analyzed with ANOVA. Separate analyses were conducted for each *O. nubilalis* generation. Corn treatment was a fixed effect, whereas year, farm, and block were treated as random effects. ANOVA also was used to analyze number of egg masses, size of egg mass, and number of larvae. Distance (edge, 0.8, 1.5, 3.1, 6.1, and 12.2 m, which is equivalent to 0, 1, 2, 4, 8, and 16 rows) was treated as a fixed effect, and random effects were year, farm, direction, and block. Orthogonal contrasts were used to compare larval production in popcorn with field corn, field corn (0.8 m) with field corn (1.5–12.2 m), and among field corn treatments (1.5, 3.1, 6.1, and 12.2 m). ANOVA also was used to analyze survival of overwintering *O. nubilalis* larvae by year. Corn treatment and tillage were treated as fixed effects, and block was treated as a random effect. Degrees of freedom were adjusted using Satterthwaite approximation method in all

experiments. Means were separated using Tukey-Kramer mean separation procedures. Data were log-transformed when SDs were proportional to the mean (heteroscedasticity), and distributions were skewed. In addition, arc-sine transformations were applied to *B. bassiana* infection percentages.

## Results

**Popcorn Evaluations.** In 2000, when seven popcorn treatments and field corn were evaluated for second-generation *O. nubilalis* larvae, no differences were detected for mean number of larvae per plant or mean number of larvae per square meter (Fig. 1). In 2000–2002, when three popcorn treatments and field corn were compared, no differences were detected in first-generation *O. nubilalis* larval density per plant or *O. nubilalis* larval density per square meter (Fig. 2). However, numbers of second-generation *O. nubilalis* larvae per plant and per square meter were significantly higher in popcorn than field corn (Fig. 3).

Mean stage of development of *O. nubilalis* was not significantly different among the four treatments during first ( $F = 0.03$ ;  $df = 3,26.3$ ;  $P = 0.99$ ) and second ( $F = 0.62$ ;  $df = 3,5.99$ ;  $P = 0.63$ ) generations. Most



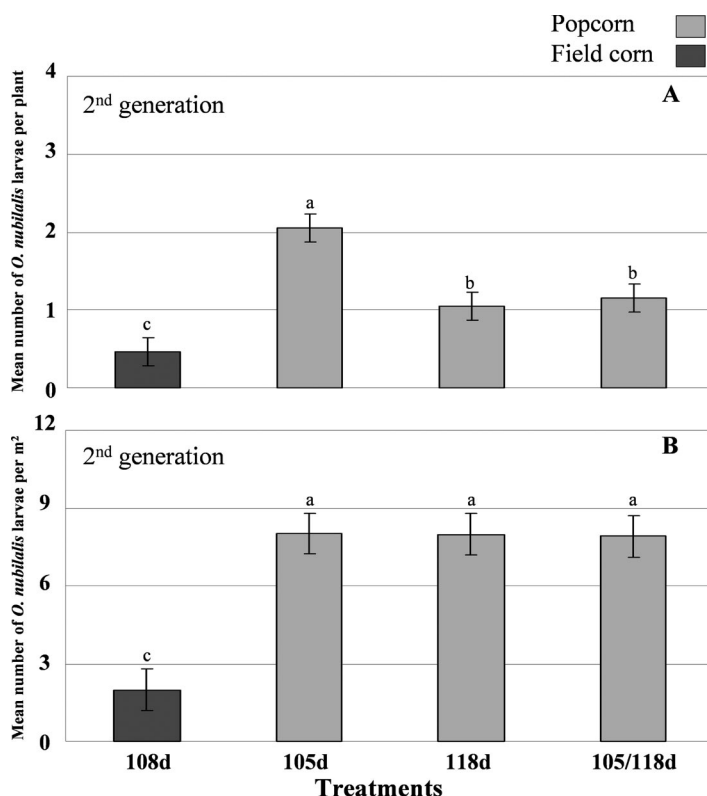


Fig. 3. (A) Mean number  $\pm$  SE of second-generation *O. nubilalis* larvae found per plant in popcorn and field corn 2000–2002. Treatment means were significantly different ( $F = 2.55$ ;  $df = 3,27$ ;  $P = 0.05$ ). (B) Mean number  $\pm$  SE of second-generation *O. nubilalis* larvae found per square meter in popcorn and field corn 2000–2002. Treatment means were significantly different ( $F = 8.77$ ;  $df = 3,6$ ;  $P = 0.01$ ). Bars marked with the same letter are not significantly different (ANOVA,  $P \leq 0.05$ ; Tukey-Kramer,  $\alpha = 0.05$ ). Day is represented by d.

larvae sampled during each generation were fourth or fifth instars.

**Popcorn and Field Corn Proximity Experiments.** Mean number of *O. nubilalis* egg masses found on corn plants differed depending on the distance from popcorn plots. Significantly more *O. nubilalis* egg masses were found on popcorn than field corn (Fig. 4A). Orthogonal contrasts indicate significantly more *O. nubilalis* egg masses were found on popcorn at 0 m than field corn at 0.8–12.2 m (Table 3). No significant differences in mean number of *O. nubilalis* egg masses were found on field corn at 0.8 m compared with field corn at 1.5–12.2 m. No significant differences in mean number of *O. nubilalis* egg masses were found among corn plants at 1.5, 3.1, 6.1, and 12.2 m away from popcorn.

Size of *O. nubilalis* egg masses also significantly differed depending on their proximity to popcorn (Fig. 4B). Significantly more *O. nubilalis* eggs per egg mass were found on popcorn plants than on field corn plants further than 0.8 m from popcorn plots. Orthogonal contrasts indicate significantly more *O. nubilalis* eggs per egg mass were found on popcorn plants than field corn at 0.8–12.2 m (Table 3). No significant differences in mean number of *O. nubilalis* eggs per egg mass were found on field corn at 0.8 m compared with

field corn at 1.5–12.2 m. No significant differences in number of *O. nubilalis* eggs per egg mass on field corn plants were observed among corn plants at 1.5, 3.1, 6.1, and 12.2 m away from popcorn.

Mean number of *O. nubilalis* larvae found in corn plants differed depending on distance from popcorn plots (Fig. 4C). Significantly more larvae per plant were found in popcorn than field corn. Field corn plants 0.8 m from popcorn also had significantly more *O. nubilalis* larvae than field corn at distances further away from popcorn. Orthogonal contrasts indicated significantly more *O. nubilalis* larvae were found in popcorn than field corn 0.8–12.2 m (Table 3). Significantly more *O. nubilalis* larvae were found in field corn plants at 0.8 m than field corn at 1.5–12.2 m away from popcorn. No significant differences in mean number of *O. nubilalis* larvae were found in field corn plants at 1.5, 3.1, 6.1, and 12.2 m away from popcorn.

**Small Field Experiments.** Mean numbers of overwintering *O. nubilalis* larvae surviving in popcorn and field corn plots were significantly different each of the 3 yr (Table 4), with popcorn to field corn advantages of  $17.1 \times$  (2001),  $18.7 \times$  (2002), and  $2.2 \times$  (2003). Larval survival in the two tillage treatments was not significantly different any of the 3 yr (Table 4).

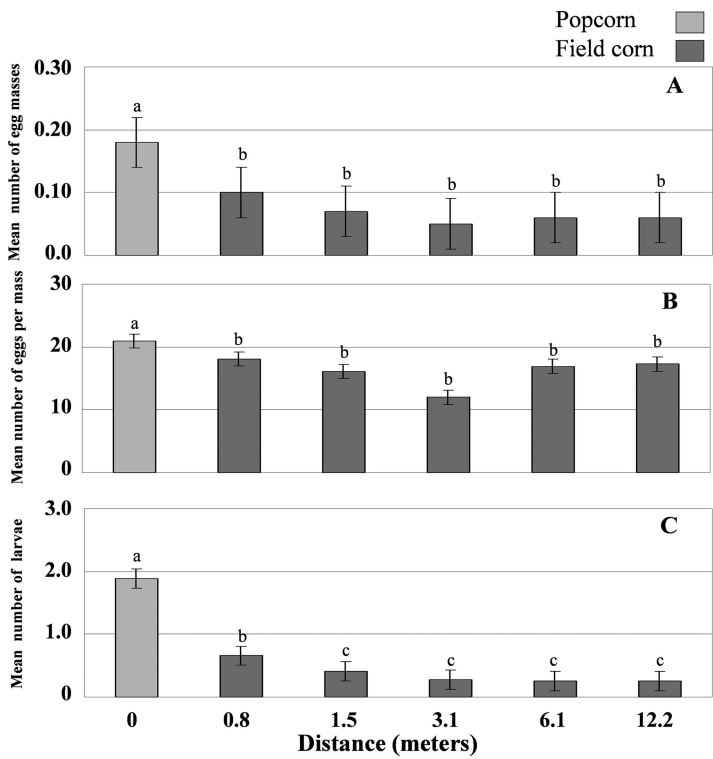


Fig. 4. (A) Mean number  $\pm$  SE of *O. nubilalis* egg masses per plant on popcorn and field corn (2000–2002;  $F = 6.50$ ;  $df = 5,150$ ;  $P = 0.0001$ ). (B) Mean number  $\pm$  SE of *O. nubilalis* eggs per egg mass per plant on popcorn and field corn (2000–2002;  $F = 3.98$ ;  $df = 5,141$ ;  $P = 0.002$ ). (C) Mean number  $\pm$  SE of *O. nubilalis* larvae per plant on popcorn and field corn (2000–2002;  $F = 42.2$ ;  $df = 5,151$ ;  $P = 0.0001$ ). Sampled field corn plants were 0.8, 1.5, 3.1, 6.1, and 12.2 m from popcorn plots (0 m). Bars marked with the same letter are not significantly different (ANOVA,  $P \leq 0.05$ ; Tukey-Kramer,  $\alpha = 0.05$ ).

Mean percentage of *O. nubilalis* larvae infected with *B. bassiana* was not significantly different in popcorn and field corn plots in 2001 and 2002 (Table 5). In 2003, however, significantly higher percentages of larvae were infected with *B. bassiana* in field corn plots compared with popcorn plots. Mean percentage of *O. nubilalis* larvae with *B. bassiana* in the no-till and conventional tillage treatments were not significantly different during any of the 3 yr (Table 5). Over the 3

yr, only 47 *M. cingulum* were found in the 1,017 *O. nubilalis* larvae that were evaluated. Although mean percentage of larvae parasitized with *M. cingulum* was significantly higher in popcorn ( $0.33 \pm 0.04$ ) than field corn ( $0.01 \pm 0.04$ ) in 2001, no significant differences in mean numbers of larvae parasitized were observed in 2002 (popcorn:  $0.03 \pm 0.01$ ; field corn:  $0.01 \pm 0.01$ ) and 2003 (popcorn:  $0.09 \pm 0.03$ ; field corn:  $0.06 \pm 0.03$ ).

Table 3. Orthogonal contrast used to compare mean numbers of *O. nubilalis* egg masses, eggs per egg mass, and larvae found on/in popcorn and field corn plants at various distances from popcorn

|   | NDF | DDF | F      | P       |
|---|-----|-----|--------|---------|
| Contrast (egg masses)                         |     |     |        |         |
| Popcorn 0 m versus field corn 0.8–12.2 m      | 1   | 150 | 27.80  | <0.0001 |
| Field corn 0.8 m versus field corn 1.5–12.2 m | 1   | 150 | 2.93   | 0.0890  |
| Field corn 1.5, 3.1, 6.1, 12.2 m              | 3   | 150 | 1.19   | 0.7356  |
| Contrast (eggs per egg mass)                  |     |     |        |         |
| Popcorn 0 m versus field corn 0.8–12.2 m      | 1   | 141 | 12.15  | 0.0007  |
| Field corn 0.8 m versus field corn 1.5–12.2 m | 1   | 138 | 2.93   | 0.0894  |
| Field corn 1.5, 3.1, 6.1, 12.2 m              | 3   | 140 | 2.49   | 0.0625  |
| Contrast (larvae)                             |     |     |        |         |
| Popcorn 0 m versus field corn 0.8–12.2 m      | 1   | 151 | 188.48 | <0.0001 |
| Field corn 0.8 m versus field corn 1.5–12.2 m | 1   | 151 | 19.11  | <0.0001 |
| Field corn 1.5, 3.1, 6.1, 12.2 m              | 3   | 151 | 1.19   | 0.3167  |

Sampled field corn plants were 0.8, 1.5, 3.1, 6.1, and 12.2 m from popcorn plot for 2000, 2001, and 2002. NDF, numerator degrees of freedom; DDF, denominator degrees of freedom.



**Table 4.** Mean  $\pm$  SE no. of *O. nubilalis* larvae surviving winter per square meter in field corn and popcorn plots and in no-till and conventional tillage plots (2001–2003)

| Year | Treatment (C) | Tillage (T)  | Mean $\pm$ SE  | Corn  |      |      | Tillage |      |      | C $\times$ T <sup>a</sup> |      |      |
|------|---------------|--------------|----------------|-------|------|------|---------|------|------|---------------------------|------|------|
|      |               |              |                | F     | df   | Pr   | F       | df   | Pr   | F                         | df   | Pr   |
| 2001 | Field corn    | Conventional | 0.56 $\pm$ 4.1 | 10.7  | 1, 6 | 0.02 | 0.02    | 1, 6 | 0.9  | 0.04                      | 1, 6 | 0.85 |
|      |               | No-till      | 0.78 $\pm$ 4.1 |       |      |      |         |      |      |                           |      |      |
|      | Popcorn       | Conventional | 12.0 $\pm$ 4.1 | 5.99  | 1, 6 | 0.05 | 0.12    | 1, 6 | 0.74 | 0.18                      | 1, 6 | 0.68 |
|      |               | No-till      | 10.9 $\pm$ 4.1 |       |      |      |         |      |      |                           |      |      |
| 2002 | Field corn    | Conventional | 0.78 $\pm$ 5.3 | 11.06 | 1, 8 | 0.01 | 0.74    | 1, 8 | 0.42 | 0.33                      | 1, 8 | 0.58 |
|      |               | No-till      | 0.44 $\pm$ 5.3 |       |      |      |         |      |      |                           |      |      |
|      | Popcorn       | Conventional | 9.70 $\pm$ 5.3 |       |      |      |         |      |      |                           |      |      |
|      |               | No-till      | 13.1 $\pm$ 5.3 |       |      |      |         |      |      |                           |      |      |
| 2003 | Field corn    | Conventional | 10.8 $\pm$ 3.6 | 11.06 | 1, 8 | 0.01 | 0.74    | 1, 8 | 0.42 | 0.33                      | 1, 8 | 0.58 |
|      |               | No-till      | 9.80 $\pm$ 3.6 |       |      |      |         |      |      |                           |      |      |
|      | Popcorn       | Conventional | 24.7 $\pm$ 3.6 |       |      |      |         |      |      |                           |      |      |
|      |               | No-till      | 19.6 $\pm$ 3.6 |       |      |      |         |      |      |                           |      |      |

<sup>a</sup> C  $\times$  T is the interaction between corn and tillage treatments.

## Discussion

A large number of *O. nubilalis* moths should be produced in refuge corn, and these moths must be available to mate with possible resistant moths during each generation. Planting, management, and harvest of alternative corn refuges such as popcorn also should be compatible with existing practices and equipment and should be agronomically compatible and affordable. Can popcorn satisfy these criteria?

In these studies, popcorn produced more *O. nubilalis* larvae than field corn, up to 4.6 and 18.7 times more in small plots and small fields, respectively. In small plots, *O. nubilalis* production from popcorn treatments (105d, 118d, and mixture) compared with the field corn was higher in the second generation of *O. nubilalis* than the first. There also was some advantage in planting mixed maturity hybrids or a long maturity hybrid. During the first generation, 1.3 times more larvae per square meter were found in the popcorn mixture (105 d and 118 d) and 1.8 times more in later maturing popcorn than in field corn. More *O. nubilalis* larvae per square meter also were found in the mixture (3.9 times) and in the later maturing popcorn (4.0 times) than in field corn during the second generation. High numbers of larvae in popcorn may be caused by a lack of resistance factors. From 299

popcorn accessions, Wilson et al. (1993) found only 14 varieties of popcorn with any resistance to whorl feeding by first-generation *O. nubilalis*. Also, no sheath or collar resistance to second-generation *O. nubilalis* feeding has been documented for popcorn (Jarvis and Guthrie 1980).

The possibility of reduced area for refuge for any GE crops raises questions about refuge isolation and the distribution of moths over a landscape (Caprio et al. 2004). This is a concern because relative abundance, temporal distribution, and spatial distribution of the *Bt* crop and refuge influences how movement rates expedite or delay resistance (Sisterson et al. 2005). With *Bt* corn and *O. nubilalis* there is the potential for refuge moth populations to be reduced if most of the moths oviposit in *Bt* corn. These studies, however, suggest oviposition is higher and egg mass size larger in popcorn than field corn, which would mitigate a possible sink effect from *Bt* corn if high proportions of the larvae survive.

Increased oviposition in popcorn may indicate *O. nubilalis* females prefer popcorn over field corn. Female preference for popcorn may be caused by pollen abundance, attractant volatiles, or a lack of repellent chemicals (i.e., antixenosis factors). Binder and Robbins (1996) determined that egg mass size decreased with female age, which suggests younger females are

**Table 5.** Mean  $\pm$  SE percentages of *O. nubilalis* larvae infected with *B. bassiana* (2001–2003)

| Year | Treatment (C) | Tillage (T)  | Mean $\pm$ SE | Corn |      |      | Tillage |      |      | C $\times$ T <sup>a</sup> |      |      |
|------|---------------|--------------|---------------|------|------|------|---------|------|------|---------------------------|------|------|
|      |               |              |               | F    | df   | Pr   | F       | df   | Pr   | F                         | df   | Pr   |
| 2001 | Field corn    | Conventional | 22 $\pm$ 4    | 0.07 | 1, 6 | 0.80 | 0.01    | 1, 6 | 0.96 | 0.01                      | 1, 6 | 0.96 |
|      |               | No-till      | 21 $\pm$ 4    |      |      |      |         |      |      |                           |      |      |
|      | Popcorn       | Conventional | 18 $\pm$ 4    | 0.01 | 1, 8 | 0.95 | 0.66    | 1, 8 | 0.44 | 1.11                      | 1, 8 | 0.32 |
|      |               | No-till      | 19 $\pm$ 4    |      |      |      |         |      |      |                           |      |      |
| 2002 | Field corn    | Conventional | 0             | 10.9 | 1, 8 | 0.01 | 0.14    | 1, 8 | 0.72 | 0.66                      | 1, 8 | 0.44 |
|      |               | No-till      | 28 $\pm$ 8    |      |      |      |         |      |      |                           |      |      |
|      | Popcorn       | Conventional | 10 $\pm$ 8    |      |      |      |         |      |      |                           |      |      |
|      |               | No-till      | 6 $\pm$ 8     |      |      |      |         |      |      |                           |      |      |
| 2003 | Field corn    | Conventional | 35 $\pm$ 1    | 10.9 | 1, 8 | 0.01 | 0.14    | 1, 8 | 0.72 | 0.66                      | 1, 8 | 0.44 |
|      |               | No-till      | 32 $\pm$ 1    |      |      |      |         |      |      |                           |      |      |
|      | Popcorn       | Conventional | 12 $\pm$ 1    |      |      |      |         |      |      |                           |      |      |
|      |               | No-till      | 18 $\pm$ 1    |      |      |      |         |      |      |                           |      |      |

<sup>a</sup> C  $\times$  T is the interaction between corn and tillage treatments.

ovipositing on popcorn. Further studies could determine the age of ovipositing females and possible corn variety and landscape influences on oviposition.

Significantly more *O. nubilalis* larvae found in the field corn row adjacent to popcorn than in subsequent rows raises concerns with *Bt* corn and popcorn interfaces. Early and late instars could move across these interfaces and violate the high-dose strategy if larvae with some genetic resistance survive (Onstad and Guse 1999, Davis and Onstad 2000). However, such concerns would be reduced or eliminated if popcorn is planted in separate fields or blocks. Separate plantings may be necessary anyway because there are agronomic incompatibilities with popcorn and field corn.

An effective popcorn refuge also must have high survival of *O. nubilalis* larvae during the winter and through spring until adult emergence. In the small-field studies, significantly more larvae overwintered in popcorn than field corn during all years (2001–2003), from 2.2 to as many as 18.7 times more larvae. This suggests larger populations of *O. nubilalis* moths from the popcorn would be available to mate during the first generation flight the following year.

A decrease of the popcorn refuge advantage occurred in 2003 compared with the 2 previous yr. In this case, it seems that the *O. nubilalis* production in field corn increased at a faster rate than that of popcorn. Perhaps this is related to density-dependent variables, e.g., high numbers of *O. nubilalis* (eggs, larvae, and adults) and associated damage from larvae in the popcorn may have deterred moth oviposition in the popcorn. Another possibility is increased infection rates with entomopathogens (e.g., *B. bassiana*) in popcorn, which could reduce population density. *B. bassiana* is an important fungal pathogen of *O. nubilalis* (Bartlett and Lefebvre 1934). In these studies, percentages of *O. nubilalis* larvae infected with *B. bassiana* were significantly higher in field corn plots than popcorn plots only in 2003. This suggests that *B. bassiana* has the potential to increase in corn fields when refuge is located in the same area year after year or as *O. nubilalis* density increases. Further studies are needed to clarify this point, but possible impacts of *B. bassiana* on *O. nubilalis* should not be ignored when considering long-term refuge strategies.

Production, recruitment, and retention of *O. nubilalis* in popcorn refuge are evident, but popcorn and field corn could be viewed as incompatible. This incompatibility is because of an allele (Gal-s) that controls cross-pollination of these two subspecies. Receptivity of field corn and popcorn silks to pollen from other subspecies ranges from completely receptive to nonreceptive (Nelson 1952). Field corn silks are completely receptive to cross-pollination with popcorn pollen, which could result in xenia (Poehlman 1987) and reduced grain quality in the *Bt* corn. In addition, potential reductions in refuge yield and grain quality may reduce the economic value of refuge corn. Therefore, it may be more realistic to use a “sacrificial” refuge. However, growers may be opposed to sacrificing the acreage needed for a refuge. Further study

is needed to determine how much moth production from refuge is needed to economically justify a “sacrificial” refuge. A large production of moths, after careful consideration of refuge effectiveness, could justify reducing refuge size and at some point could become economical for growers.

Approval of *Bt* corn for control of corn rootworms, *Diabrotica* spp., and development of stacked-trait hybrids effective against both *O. nubilalis* and *Diabrotica* beetles means further research on refuge is needed. A refuge capable of producing susceptible adults to help manage resistance in two insects is desirable. Other types of corn, including different accessions of popcorn and corn with tropical germplasm could be evaluated for potential as refuge plants. These questions should be answered before any type of corn can be considered a viable refuge alternative to non-*Bt* field corn. Once these questions are answered refuge design, planting and maintenance could develop into a cottage industry, whereby specialists could help individual growers or, on an areawide basis, a group of growers satisfy their refuge requirements.

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